CELL ANATOMY

BY ROBERTA KWOK

A menagerie of intriguing cell structures, some long-neglected and others newly discovered, is keeping biologists glued to their microscopes.

In 2008, Chalongrat Noree faced an unenviable task: manually surveying hundreds of yeast strains under a microscope. Each strain had a different protein tagged with a fluorescent label, and Noree, a graduate student at the University of California, San Diego, was looking for interesting structures in the cells.

But it wasn't long until Noree's labour yielded results: within a month, he began finding a wide variety of proteins assembling into clusters or long strands. "Imagine every week you found a new intracellular structure," says Jim Wilhelm, a cell biologist and Noree's adviser. "If it were a slot machine, it would be paying off every other time you pulled the handle."

These days, textbook diagrams of cell structures such as the nucleus, mitochondrion, ribosome and Golgi apparatus are beginning to seem out of date. New imaging techniques, genome data, interest from disciplines outside cell biology and a bit of serendipity are drawing attention to an intricate landscape of tubes, sacs, clumps, strands and capsules that may be involved in everything from intercellular communication to metabolic efficiency. Some could even be harnessed for use in drug delivery or in synthesis of industrial products, such as biofuels.

Some of these structures have been known for decades, whereas others have only recently come to light. Wilhelm's team, for instance, has found six kinds of filament that either had never been described, or had been largely passed over. "You figure, how many structures could have been missed in the cell?" says Wilhelm. "Apparently, a lot more than you would imagine."

LINES OF COMMUNICATION

One structure that is receiving fresh scrutiny is the membrane nanotube: a thin thread of membrane suspended between cells. In 2000, Amin Rustom, then a graduate student at Heidelberg University in Germany, was using a newly acquired dye to look at rat tumour cells under a fluorescence microscope. But he decided to skip some washing steps in the protocol. "He said, 'I saw something — I don't know what it is, but it looks interesting,'" recalls his former adviser, Hans-Hermann Gerdes, a cell biologist now at the University of Bergen in Norway. The tubes that Rustom had noticed were so straight that Gerdes initially wondered if they were scratches on the dish.

The team concluded in a 2004 study[1] that the structures, which could span the distance of several cells, were channels that could transport small cellular organelles. That same year, Daniel Davis, a molecular immunologist at Imperial College London, and his colleagues proposed that immune cells might send signals to each other along such tubes[2]. At the time, Davis recalls, "There would always be people in the audience who would say, 'I saw those strands in the late 1970s or 80s'." But earlier observers paid little heed to the tubes.

The 2004 reports prompted more studies, which have found nanotubes in many types of mammalian cell. Davis's team found that nanotubes could help certain white blood cells to kill cancer cells, either by acting as a tether that draws the cancer cell close or by providing a conduit for delivering lethal signals[3]. Nanotubes can also conduct electrical signals,

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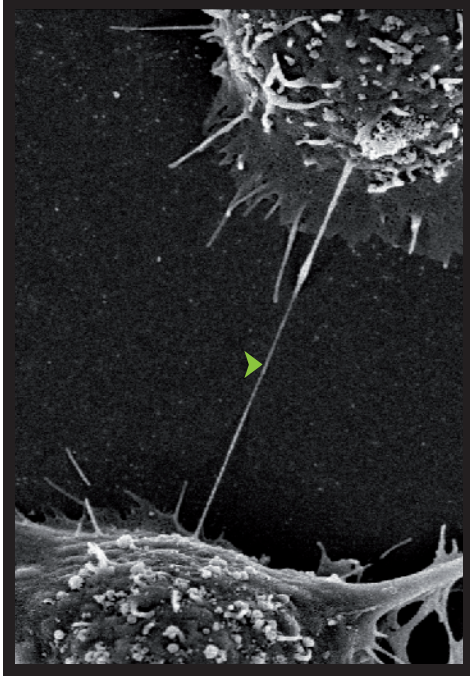
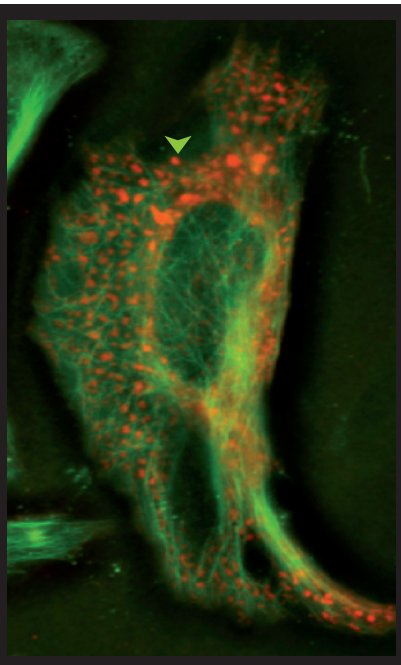
(FROM LEFT) REF. 1; REF. 8; S. TANAKA *ET AL. SCIENCE* **319**, 1083–1086 (2008)

LINES OF COMMUNICATION

Nanotubes are thin membranous threads that run between cells and may serve as signalling conduits.



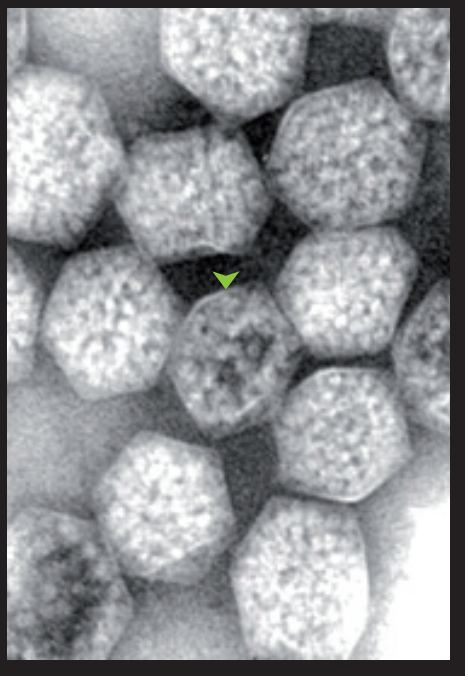


PRODUCTIVITY HOTSPOTS

Some metabolic enzymes clump together under certain conditions, possibly to improve their efficiency.

TINY FACTORIES

Orderly microcompartments seem to cordon off certain chemical reactions from the rest of the cell.



which might enable cells to coordinate during migration or wound healing, according to a 2010 study by Gerdes and his colleagues[4]. HIV and prions — infectious, misfolded proteins — may even travel along the tubes[5,6].

Some researchers are sceptical that nanotubes can form open channels. "It's not clear that there's a real continuous tunnel," says Jennifer Lippincott-Schwartz, a cell biologist at the US National Institutes of Health in Bethesda, Maryland. And so far, nanotubes have been studied mainly in cell culture. Blocking nanotube formation in living organisms might give clues to their importance, says Davis. But such manipulations often disturb other crucial processes.

PRODUCTIVITY HOTSPOTS

Researchers have long puzzled over how some metabolic processes work so efficiently. If the proteins involved are not close together, intermediate molecules could get lost in the "bewildering mass of enzymes in the cell", says Stephen Benkovic, a chemical biologist at Pennsylvania State University in University Park. Proteins often assemble to carry out a particular task — a large complex is required to copy DNA, for example — but Benkovic and others have wondered whether metabolic enzymes might cluster together in a multistep assembly line, passing sometimes-unstable molecules from one 'worker' to the next.

Benkovic's group found evidence that this clustering does occur in enzymes that produce a precursor of purine nucleotides, which are components of DNA and RNA. The team tagged each enzyme with a fluorescent label and observed them in living cells under the microscope. When a cell was deprived of purines, the enzymes grouped together in a cluster, which the team called the 'purinosome'[7]. Last year, the team reported that purinosomes are nestled in a mesh of protein fibres called microtubules, like berries in a bramble bush[8]. The molecules produced by purinosomes can be converted to the cellular fuel adenosine triphosphate, so Benkovic speculates that purinosomes may help power the transport of organelles and materials around the cell on microtubule tracks.

Edward Marcotte, a systems biologist at the University of Texas at Austin, advises caution in interpreting these results, however. He and his colleagues have seen enzyme clusters as well: in 2009, they reported that they had found 180 types of protein forming clumps in starved yeast cells[9]. But it is not clear whether the clumps serve a useful purpose — such as improving metabolic efficiency or acting as storage depots — or are a result of cellular failures brought on by starvation, says Marcotte.

TINY FACTORIES

Some researchers are taking a closer look at elegant bacterial protein containers called microcompartments. First seen about 50 years ago, these polyhedron-shaped protein capsules resemble the outer shell of a virus[10]. But unlike viruses, which package genetic material, microcompartments contain enzymes that carry out important reactions, such as converting carbon dioxide into a form of carbon that is usable by the cell. Scientists suspect that the shells make reactions more efficient, keep toxic intermediate products away from the rest of the cell and protect enzymes from molecules that could hinder their performance.

In 2005, protein crystallographers helped to reveal the capsules' finer details. Microcompartments "simply hadn't attracted the attention yet of structural biologists", says Todd Yeates, a structural biologist himself at the University of California, Los Angeles. He and his colleagues found that some shell proteins assemble into six-sided tiles that come together to form the sides of a microcompartment[11]. Each tile has a hole in the centre that could allow molecules to pass through.

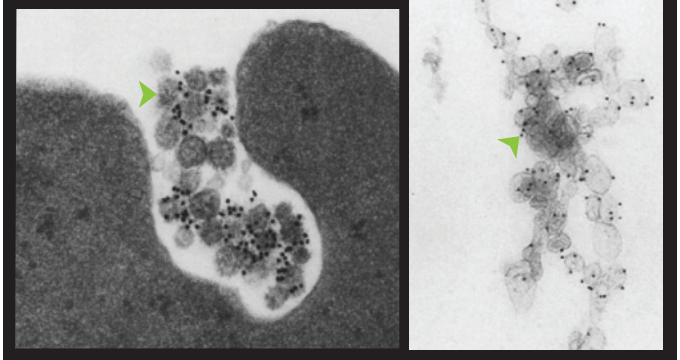
In addition to having an orderly structure, microcompartments can also line up in neat rows. Pamela Silver, a synthetic biologist at Harvard Medical School in Boston, Massachusetts, and her colleagues reported[12] last year that in cyanobacteria, certain microcompartments called carboxysomes "more or less stayed in a line down the centre of the cell", says Silver. This tidy arrangement allows cells to allot carboxysomes evenly to daughter cells when dividing.

Biologists are now eager to exploit these capsules for industrial uses by loading them with different enzymes. For instance, Yeates and his team are planning to try engineering microcompartments to produce biofuel. Some researchers have managed to package fluorescent proteins or enzymes from other species into the shells, suggesting that it is possible to modify the capsules' contents.

Microcompartments still offer plenty of unexplored territory. Scientists aren't sure, for instance, exactly how enzymes are organized

CARGO CONTAINERS

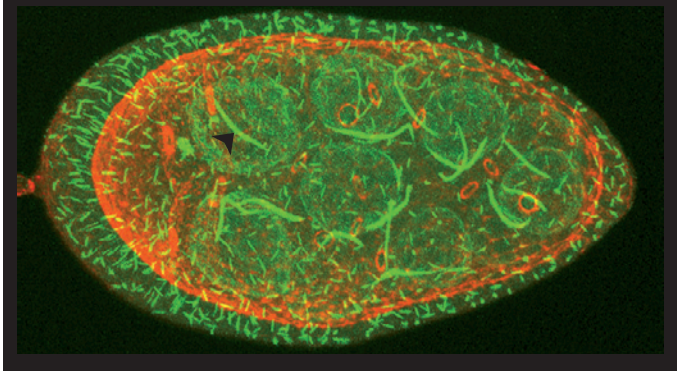
Exosomes (seen here in two different environments) can parcel up and distribute molecules to distant cells.



(LEFT) B.-T. PAN *ET AL. J. CELL BIOL.* **101,** 942–948 (1985); REF. 13

CELL SERPENTS

Some enzymes form filaments that serve as structural scaffolding and may control reactions en masse.



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inside the capsules, says Cheryl Kerfeld, a structural biologist at Lawrence Berkeley National Laboratory in Berkeley, California. "We don't really know what it looks like in there."

CARGO CONTAINERS

Other subcellular packages drawing attention are exosomes — tiny membrane-enclosed sacs that form inside the cell and are later spat out. These nanoscale vessels were discovered in the 1980s and then ignored for about a decade — considered a way of bagging up cellular rubbish. "People thought they were junk, basically," says Jan Lötvall, a clinical allergist at the University of Gothenburg in Sweden.

Interest in exosomes picked up in 1996, when Graça Raposo, a cell biologist now at the Curie Institute and the National Centre for Scientific Research in Paris, and her colleagues scrutinized exosomes spat out by B cells, a type of white blood cell. Although the technology to examine them — electron microscopy — wasn't new, it wasn't very popular at the time because "it was just old-fashioned", says Raposo. Using it and other techniques, the team reported that the humble vessels might do something useful: display scraps of pathogen protein on their surfaces, spurring immune cells to mount defences against an infection[13]. Scientists became even more intrigued when Lötvall's team reported in 2007 that exosomes could carry messenger RNA[14], some of which could be picked up and translated in a recipient cell. This suggested that the shipments might allow cells to affect protein production in their neighbours. The study "really showed that exosomes were a vehicle of communicating important information between cells", says Clotilde Théry, a cell biologist who is also at the Curie Institute.

Researchers are now trying to use exosomes to deliver drugs to specific parts of the body — with the hope that, because exosomes are 'natural', they might be less likely to be toxic or provoke an immune response than other vessels, such as artificial lipid sacs or protein shells. This year, Matthew Wood, a neuroscientist at the University of Oxford, UK, and his colleagues reported[15] an attempt in mice: the team loaded exosomes with artificial RNA intended to hinder production of a protein involved in Alzheimer's disease and tagged them with a molecule directing them to neurons and the blood–brain barrier. The exosomes successfully delivered their cargo and reduced production of the protein with no obvious ill effects, the team found. Other scientists are trying to fish exosomes out of body fluids and analyse their contents to diagnose cancer or deploy exosomes to provoke immune responses against tumours.

CELL SERPENTS

Finally, Wilhelm's group and others have found filaments that string together enzymes by the hundreds or thousands — enough, in some cases, to span nearly the entire cell. One of the filament-forming enzymes Wilhelm's team found was CTP synthase, which makes a building block for DNA and RNA[16]. Two other teams discovered the same filaments in fruitflies and bacteria at around the same time[17,18]. One researcher, Ji-Long Liu, a cell biologist at the Medical Research Council Functional Genomics Unit at the University of Oxford, named them cytoophidia (or 'cell serpents') because of their snake-like shapes in fly cells. Wilhelm suspects that researchers found the same filaments in the 1980s but never identified the protein.

These structures could allow the cell to turn enzymes on and off en masse, suggests Wilhelm. For instance, if the enzymes in a filament are inactive, the cell could activate all of them by dissolving the strand.

In some bacteria, enzyme filaments also seem to serve a structural purpose, somewhat like the actin filaments that are part of the cytoskeleton in more complex cells. When Zemer Gitai, a cell biologist at Princeton University in New Jersey, and his colleagues studied the structures in a comma-shaped bacterium called *Caulobacter crescentus,* they found that CTP-synthase filaments kept the cells' curvature in check. If there was too little of the enzyme, the cells curled up tightly; if there was too much, they straightened out[18].

It is not clear why curvature is important for the bacterium, says Gitai, but the findings suggest that the cells may have co-opted enzyme filaments to preserve cell shape. Researchers already suspect that actin is related to the enzyme hexokinase. It is possible that the cytoskeleton arose from filaments that originally formed to regulate the cell's metabolism, Gitai says.

Although the purpose and importance of some of these emerging structures is not yet clear, the research illustrates that the act of simply observing cells and their contents is alive and well. "A key aspect of doing great science is exploration," says Davis. "I think that there's a tremendous amount that we learn just by watching."■

Roberta Kwok *is a freelance writer in Burlingame, California.*

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